



Reference Number: 1860

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**Project Title:** Assessment of the effect of ovariectomy on diabetic and non-diabetic NOD/LtJ mice
 

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**Date:** Thursday, June 01, 2006**Sponsor:** Cytira, Inc.

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**Tentative Start Date:** Approximately four weeks after the purchase order is received.

**Confidentiality:** This protocol contains confidential or proprietary information concerning experimental design that should not be shared with a third party without the expressed written consent of The Jackson Laboratory.

**Mission Statement:** JAX® In Vivo Research Services are provided as a service to the biomedical research community, and in support of the research mission of The Jackson Laboratory. For over seventy years, scientists at The Jackson Laboratory have been conducting mouse-based biomedical research. As a result, we have accumulated a tremendous wealth of information related to husbandry, genetics and biology associated with selecting and using laboratory mice in research. Providing custom breeding and related support services enables us to gain new knowledge that is used in our research and the development of additional mouse models. The cumulative knowledge of The Laboratory is shared with researchers around the world through our scientific presentations, technical information

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services, Web-accessible databases, printed literature and publications, and training programs.

**Purpose of Study:**

The objective of this study is to assess the effects of ovariectomy in NOD mice that become diabetic at 20 weeks of age and non-diabetic NOD/LtJ mice of the same age.

**PROJECT SUMMARY**

Forty NOD/LtJ 8 week old female mice will be transferred to our *in vivo* research laboratory in West Sacramento, CA. The mice will be ear notched for identification and housed in individually and positively ventilated polycarbonate cages with HEPA filtered air at a density of 3 mice per cage. Bed-o-cob corn cob bedding will be used and cages will be changed every two weeks. The animal room is lighted entirely with artificial fluorescent lighting, with a controlled 12 h light/dark cycle (7 am to 7 pm light). The normal temperature and relative humidity ranges in the animal rooms are 22 + 4°C and 50 + 15%, respectively. The animal rooms will be set to have 15 air exchanges per hour. Filtered tap water, acidified to a pH of 2.8 to 3.2, and LabDiet 5LL4 will be provided *ad libitum*.

The study will proceed as detailed below:

1. Body weight will be monitored every two weeks to age 16 weeks and weekly thereafter
2. Diabetic state will be assessed twice per week by testing for glycosuria
  - a. A positive glycosuria test before age 16 weeks will result in the removal of the mouse from the study
  - b. After age 16 weeks mice that are positive for glycosuria will receive a follow-up test of blood glucose level by glucometer strip and mice found to have blood glucose levels  $\geq 200$  mg/dl for 2 days before age 20 weeks will be removed from the study
  - c. At age 20 weeks all non-diabetic mice and mice that become diabetic at age 20 weeks will remain in the study
3. Blood will be collected at age 20, 22, and 24 weeks and processed to serum
  - a. The serum chemistry panel will analyze:

- Albumin
- Alkaline phosphatase
- ALT (alanine transaminase)
- AST (aspartate aminotransferase)
- BUN (blood urea nitrogen)
- Calcium
- Serum chloride
- CO<sub>2</sub> (carbon dioxide)
- Creatinine
- Direct bilirubin
- Gamma-GT
- Glucose
- LDH
- Phosphorus
- Potassium
- Sodium
- Total bilirubin

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- Total cholesterol
  - Total protein
  - Uric acid
4. Food and water intake will be measured per cage from age 20 weeks to 24 weeks
  5. Clinical observations will be conducted at age 16, 20, 22, and 24 weeks, and will include gait and posture assessment as well as general condition, physical characteristics, and welfare assessment
  6. OGTT will be conducted at age 22 and 24 weeks
  7. Ovariectomies will be conducted at age 22 weeks
  8. CCMS will be conducted during age 23 and 24 weeks
  9. DEXA scan will be conducted prior to the ovariectomy and at the end of the 24th age week
  10. At the terminus of the study, the brains from 4 mice that became diabetic at age 20 weeks and 4 mice that remain non-diabetic will be harvested following whole body perfusion with 10% neutral buffered formalin
    - a. Brains will be post-fixed overnight in a 10:1 volume to brain weight formalin solution and then transferred to 70% EtOH

**DELIVERABLES**

- Deliverable 1. Tissue samples will be returned to you by courier and blood chemistry results will be provided in Excel format upon receipt from our pathology partner. JAX® In Vivo Research Services will contact George Karkanas to obtain an appropriate address for shipping data and tissue samples at the conclusion of the study.
- Deliverable 2. A final report will be provided within 45 days after study completion.

**COST ESTIMATE**

Please note: this is an estimate of project costs; any changes to the study design/protocol may result in additional charges. You will be billed on a monthly basis for actual usage of services. Alternative billing arrangements must be approved in advance of study initiation. This estimate assumes that the Sponsor will provide sufficient quantities of test articles as well as specifications regarding their use and handling.

1. Mice and housing (price for mice is not included)	\$ 2,100
2. Procedures	\$21,750
3. Project Management fees, including administrative fees, protocol development, facilities, equipment, quality assurance, health monitoring, supervisory staff, data review, and client communications	\$ 4,000

<b>TOTAL</b>	<b>\$27,850</b>
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**TIMELINES**

- The Jackson Laboratory-JAX West facility is an AAALAC accredited-facility and has rigorous IACUC review procedures to ensure the highest quality animal use and care. IACUC protocol

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development is initiated upon receipt of a purchase order by JAX® Services and approval is required prior to study initiation.

- Interim data updates during the course of the study may be provided by arrangement through the Study Director.
- Study folders containing raw data and other study-related documents will be archived by The Jackson Laboratory for 1 year. At the end of that time, you will be contacted to determine whether documents should be destroyed or shipped to you for an additional fee.
- Your samples will be disposed of unless prior arrangement for their return to you has been made. Shipping costs will be billed to you.

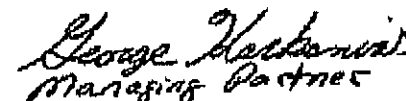
#### TERMS AND CONDITIONS

- The Jackson Laboratory will follow the protocol described in the project outline and approved by the IACUC.
- This quote expires thirty days from the issue date.
- This quote is based upon quantities stated.
- The General Terms and Conditions of the Sale are described in the JAX Mice Catalog and incorporated by reference in this estimate.
- You have the option to discontinue a project by submitting your request in writing to the Study Director. You will be billed the larger of \$400 or all charges incurred up to the date the written request is received.
- All schedules for service and delivery are approximate and based upon known factors. Unforeseen factors may impact total project time and costs.

#### APPROVAL SIGNATURES

  
Study Director, The Jackson Laboratory

6/3/2006  
Date

  
Managing Partner  
Cytira, Inc.  
Sponsor Name, Title, Organization

6/1/2006  
Date

#### STUDY INITIATION

To initiate the study without delay, please send a purchase order and a signed copy of this quote by FAX to:

JAX In Vivo Research Services  
FAX +1 916 373 6950

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